

Graphis yunnanensis (Ostropales, Graphidaceae), a New Lichen Species from China

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Abstract *Graphis yunnanensis* is described here as a new species. It is taxonomically well characterized by entire labia, slit-like disc, lateral thalline margin, completely carbonized proper exciple, inspersed hymenium, 8-spored asci, muriform 25~45 × 8~12 μm ascospores, and the presence of norstictic and stictic acids. The separation of the new species from other somewhat similar *Graphis* species is discussed. In addition, the study includes *Graphis tenoriensis* and *Phlyctis karnatakana* reported for the first time from China.

Keywords Graphidaceae, New records, Pu'er National Forest Park, Taxonomy, Yunnan

During a field excursion to China, some interesting lichen material was noticed and collected from Yunnan in December 2013 under the National Research Foundation of Korea and the Korea National Research Resource Center Program. Yunnan is a mountainous province of China designated as a global biodiversity hotspot. It is the most diverse area situated in the southwest of the country, with a humid subtropical climate. All of the collections were made in an evergreen forest in Pu'er National Forest Park in the province. The area is home to several bark-inhabiting lichens, chiefly belonging to *Graphidaceae*. Among these, an interesting *Graphis* species producing norstictic and stictic acids and characterized by entire labia, slit-like disc, lateral thalline margin, completely carbonized proper exciple, and muriform ascospores is described here as a new species. Furthermore, *G. yunnanensis* and *G. tenoriensis* are reported as new for the country. Previously, more than 100 species of the genus *Graphis* have been reported from

China, excluding *c.* 45 species that have been reported from Hong Kong [1-5].

In addition to *Graphidaceae*, patches of non-graphidaceous leprose taxa were also noticed among graphidoid members of *Hemithecium* and *Pallidogramme*, and a few of these were recognized as *Phlyctis* (*Ostropales*, *Phlyctidaceae*). This genus comprises more than 20 species worldwide that are typically characterized by the crustose, granular to leprose thalli, innate to subimmersed apothecia, a poorly developed proper exciple, muriform to transversely septate ascospores, and a thallus producing different secondary metabolites or with the rare absence of compounds [6-14]. For several years, *Phlyctis* was neglected in Chinese lichenology; however, a few recent studies have recorded *Phlyctis schizospora* Zahlbr. and *P. subargena* R. Ma & H. Y. Wang from China [10]. The current report includes a new record of *Phlyctis karnatakana* in Chinese lichen biota, which was previously known only from India (Western Ghats). The occurrence of *P. karnatakana* in Yunnan suggests connecting links between Yunnan and the Western Ghats in India. Further sampling in Yunnan can predict the extent of affinities between Chinese and Indian elements, and could reveal several undiscovered lichen components from Yunnan.

MATERIALS AND METHODS

The field excursion was organized by Dr. X. Y. Wang, Kunming Institute of Botany, CAS in December 2013. Duplicates of the specimens were deposited in the herbarium of the Korean Lichen Research Institute (KoLRI), Suncheon National University, South Korea. The taxonomy of the material was studied in the lichenology laboratory of the CSIR-National Botanical Research Institute, Lucknow, India,

Mycobiology 2015 June, 43(2): 118-121
<http://dx.doi.org/10.5941/MYCO.2015.43.2.118>
pISSN 1229-8093 • eISSN 2092-9323
© The Korean Society of Mycology

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Received April 26, 2015

Revised May 27, 2015

Accepted June 4, 2015

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using standard microscopy techniques. Morphological and anatomical details were observed under stereozoom (LEICA S8AP0; Leica Microsystems, Chennai, India) and compound (LEICA DM500; Leica Microsystems) microscopes, respectively. Relevant literature [2, 15-20] was consulted for the identification of species. The color test and thin layer chromatography in solvent systems A [toluene (180) : 1, 4-dioxane (45) : acetic acid (5)] and C [toluene (170) : acetic acid (30)] were performed following Orange *et al.* [21]. Lugol's solution (I) was used to check the amyloidity of asci and ascospores. Illustrations were prepared using CorelDRAW (version 12).

RESULTS AND DISCUSSION

Taxonomy.

Graphis yunnanensis S. Joshi, Upreti & Hur sp. nov. (Fig. 1A~1D)

Mycobank No.: MB 812736.

Differs from *Graphis saxicola* in corticolous habitat, producing 8-spored asci, and thallus containing norstictic and stictic acids.

Type: China, Yunnan Province, Pu'er City, Pu'er National Forest Park, 22°52'08.6" N, 100°59'25.5" E, alt. 1,552 m, on tree trunk, 20 Dec 2013, Hur, Wang & Liu CH130425 (holotype KoLRI 020831).

Description: Thallus corticolous, crustose, epiperidermal, verrucose, ± shiny, olivaceous, green, pale green to greyish green, up to 150 µm thick in cross section, corticate; cortex irregular, loose, 15~20 µm; photobiont layer Trentepohlioid, 50~60 µm; medulla white, crystalline, up to 80 µm, mostly endoperidermal; ascomata lirelliform, immersed to emergent; lirellae simple to rarely branched, 1~1.5 mm long; labia entire, black; disc slit-like; thalline margin lateral, 40~85 µm wide; proper exciple completely carbonized, (20~) 50~70 µm wide; epihymenium brownish, 10~15 µm high; hymenium interspersed with oil globules dissolve in K, 75~150 µm high; subhymenium hyaline to yellowish, 20~25 µm high; asci clavate, 8-spored, 75~120 × 20~25 µm; ascospores hyaline, ellipsoidal, muriform, 25~45 × 8~12 µm, 9~12 × 2~3 locular, I+ blue, halonate; halo 6~4 µm thick.

Chemistry: K+ yellow turning red, PD+ yellow-orange, C-; norstictic and stictic acids detected in thin layer chromatography (TLC).

Etymology: The specific epithet refers to the type locality in Yunnan Province, China.

Distribution and ecology: The new species is known only from the type locality in Yunnan, and collected at an elevation of c. 1,500 m, where it was growing luxuriantly among other graphidaceous taxa, including *Hemithecium*, *Diorygma*, *Pallidogramme*, *Platygramme*, and *Graphis*.

Remarks: The new species is well characterized by its corticolous habitat; mostly short, rarely branched lirellae; entire labia; slit-like disc; lateral thalline margin; completely carbonized proper exciple; densely interspersed hymenium; 8-spored asci; muriform ascospores, and thallus producing

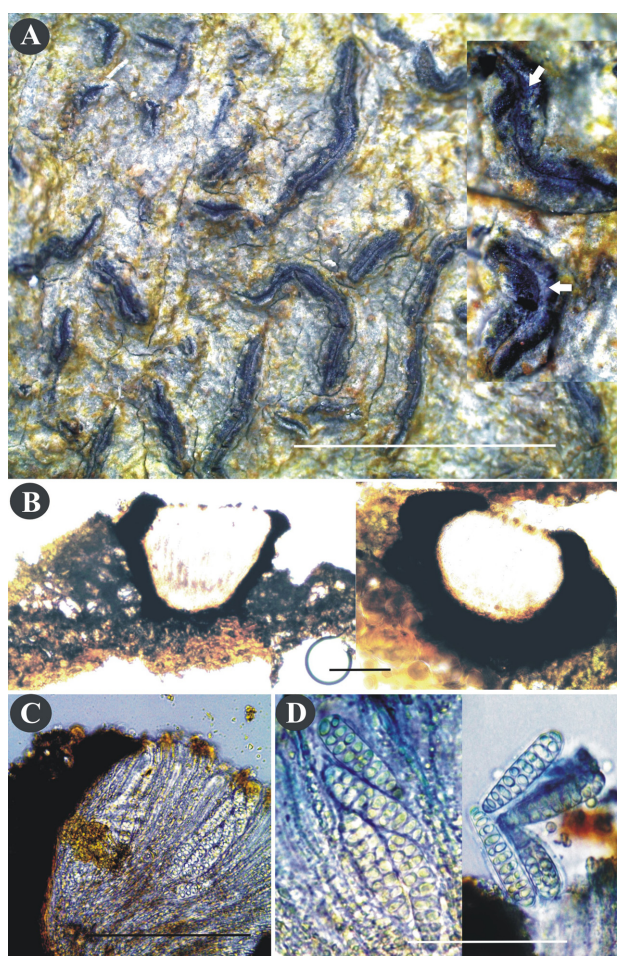


Fig. 1. *Graphis* species, habitus. A, *G. yunnanensis* (holotype), note the slightly open ascomatal disc in overmature lirellae; B, Completely carbonized proper exciple; C, Cross section of ascomata showing interspersed hymenium and 8-spored ascus; D, Ascospores (in cotton blue) (scale bars: A = 1 mm, B, D = 50 µm, C = 100 µm).

norstictic and stictic acids. The lirellae in the new species rarely show a slightly opened disc covered by a thin white layer, which might be the consequence of over-maturity or the moist conditions of the forests. *Graphis saxicola* (Müll. Arg.) A. W. Archer is most similar to *G. yunnanensis* in having entire labia, completely carbonized proper exciple, interspersed hymenium and muriform ascospores, 40~55 µm long, but differs in the thick thalline margin (*subserpentina*-morph), (2~) 4-spored asci, thallus lacking stictic acid, and the saxicolous habitat. *Graphis leprographa* Nyl., *G. novopalmiticola* A. W. Archer & Lücking, and *G. intersporectica* Sipman & Lücking also have entire labia, completely carbonized proper exciple, interspersed hymenium, and muriform ascospores, but can be distinguished in lirella morphology. In addition, *G. leprographa* and *G. novopalmiticola* have larger ascospores and thallus lacking stictic acid, while *G. intersporectica* contains only stictic acid and has restricted distribution in the Neotropics. The over-mature

lirellae of *Graphis yunnanensis* can be compared with ascomata (*scripta*-morph) of *Graphis aperiens* Müll. Arg. In addition, both species have entire labia, completely carbonized proper exciple, interspersed hymenium, and a thallus producing norstictic acid, but *G. aperiens* is easily separated in having small, transversely septate ascospores and the absence of stictic acid. *Graphis pertriosa* (Kremp.) A. W. Archer resembles the new species in having entire labia, interspersed hymenium, muriform ascospores, and the presence of norstictic acid in the thallus, but has different lirella morphology (*negrosina*-morph), laterally carbonized proper exciple, and thallus lacking stictic acid [19].

Graphis fujianensis Z. F. Zia & J. C. Wei is nearer to the new species in having entire labia, completely carbonized proper exciple, muriform ascospores, 20~50 µm long, and thallus containing stictic acid, but lirella morphology (*hossei*-morph), clear hymenium, and absence of norstictic acid clearly distinguish it from *G. yunnanensis* [15, 19].

Specimens examined: China, Yunnan Province, Pu'er City, Pu'er National Forest Park, 22°52'08.6" N, 100°59'25.5" E, alt. 1,552 m, on tree trunk, 20 Dec 2013, Hur, Wang & Liu, CH130423 (KoLRI 020829), CH130425 (holotype KoLRI 020831).

***Graphis tenoriensis* Lücking & Chaves (Fig. 2A)**

Fieldiana, Bot. 38: 115 (2008).

Description: Thallus corticolous, crustose, epiperidermal, continuous, slightly verrucose, shiny, greenish gray to gray, corticate; cortex 15~20 µm thick; photobiont layer Trentepohlioid, 30~40 µm thick; medulla 50 µm to mostly endoperidermal, crystalline; ascomata lirelliform, emergent; lirellae elongate, irregularly branched, 0.5~1 × 0.02~0.03 mm; disc concealed; labia 1~2-striate; thalline margin lateral 40~45 µm thick; proper exciple apically carbonized, 70~120 µm thick; epihymenium pale brown, 5~10 µm high; hymenium hyaline, clear, 100~120 µm high; subhymenium hyaline, 25~30 µm high; asci 1-spored, broadly clavate, 85~110 × 25~35 µm; ascospores hyaline, oval, muriform, 100~110 × 28~30 µm, halonate.

Chemistry: K–, PD–, C–; no lichen compound detected in TLC.

Distribution and ecology: Central America [19]; in China, the species was collected between 1,500 and 1,600 m from thick-barked trees, where it was growing luxuriantly with other graphidoid taxa.

Remarks: The species is similar to *Graphis platycarpa* Eschw., which differs in apically thin complete thalline margin, 8-spored asci, and small ascospores [19].

Specimens examined: China, Yunnan Province, Pu'er City, Pu'er National Forest Park, 22°35'53" N, 101°06'30.2" E, alt. 1,590 m, on tree trunk, 18 Dec 2013, JS Hur, XY Wang and D Liu, CH130297 (KoLRI 020703), CH130298 (KoLRI 020704).

***Phlyctis karnatakana* S. Joshi & Upreti (Fig. 2B and 2C)**
Bryologist 113: 726 (2010).

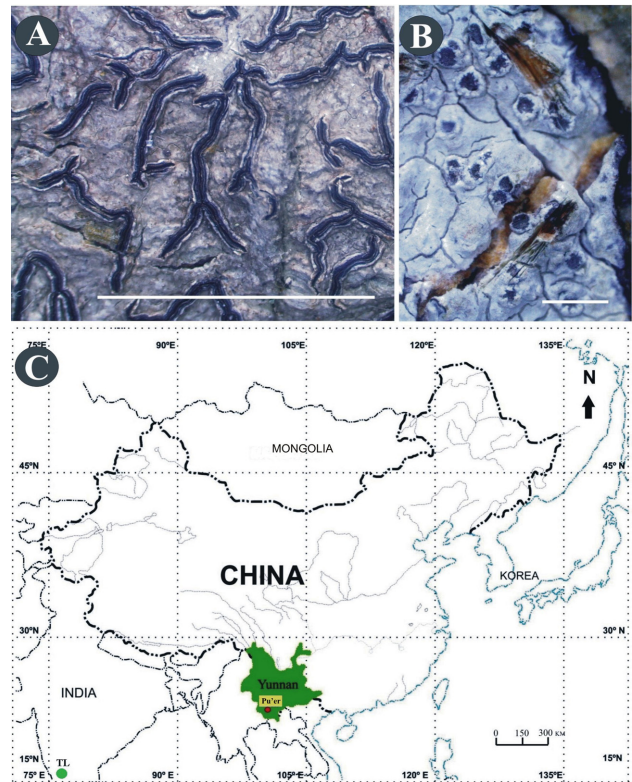


Fig. 2. A, Habit of *Graphis tenoriensis*; B, Habit of *Phlyctis karnatakana*; C, Map showing type locality (TL-Shimoga district, Karnataka) of *Phlyctis karnatakana* in India, and locality surveyed (Pu'er, Yunnan) in China (scale bars: A, B = 1 mm).

Description: Thallus corticolous, crustose, epiperidermal, subleprose, ± rimose due to substrate, greenish grey, whitish green to greyish green, 100~150 µm thick in cross section, ecorticate; photobiont layer of green protococcoid alga, 40~60 µm; medulla white, mostly endoperidermal, up to 90 µm; prothallus white; ascomata round to irregular, solitary to aggregate, immersed, 0.4~0.5 mm, disc black, finely pruinose, 0.2~0.3 mm in diameter; thalline margin recurved, 165~180 µm wide; proper exciple hyaline, apically dark, 30~50 µm wide; epihymenium granular, brown, 10~15 µm high; hymenium hyaline, clear, 130~140 µm high, I+ wine red; hypothecium brown to dark brown, ≤ 30 µm high; asci clavate, 8-spored, 100~120 × 10~15 µm, I+ wine red; ascospores hyaline, fusiform, crescent shaped, transversely septate, 25~35 × 4~7 µm, 8-locular, I+ wine red.

Chemistry: K+ yellow turning red, PD+ yellow-orange, C–; norstictic acid detected in TLC.

Distribution and ecology: This is the second record of *Phlyctis karnatakana* from China. Previously, the species was described from the Western Ghats in India [11, 14]; in China the species was growing in close association with *Pallidogramme chlorocarpoides* and distributed among species of *Hemitecium*, *Diorygma*, and *Chapsa*, and was collected from thin-barked trees in a forest in a national park.

Remarks: This species is close to *Phlyctis uncinata* (Stirton) Müll. Arg., which also produces transversely septate ascospores, and norstictic acid in the thallus, but differs in large-sized ascospores having greater septation. *Phlyctis longifera* (Nyl.) D. J. Galloway resembles *P. karnatakana* in having 7-septate ascospores, but differs in ascospore size (55~86 × 5~7 µm) and the presence of stictic acid in the thallus [6, 13]. *Phlyctis communis* Chitale & Makhija also contains norstictic acid and produces transversely septate ascospores, but differs from *Phlyctis karnatakana* in producing salazinic acid additionally and 7~16-septate ascospores. As discussed [13], the genus *Phlyctis* contains several taxonomic disparities in species delimitation. Indeed, the current status of the genus relies apparently on differences in habitat, thallus chemistry, and slightly varied thallus morphology. In some cases, species delimitation is also biased in ascospore septation, which has little significance in major taxonomic variation [22]. Worldwide monographic studies supported by molecular analysis could improve species delimitations and phylogenetic relationships within the genus [13].

Specimen examined: China, Yunnan Province, Pu'er City, Pu'er National Forest Park, 22°52'08.6" N, 100°59'25.5" E, alt. 1,552 m, on bark, 20 Dec 2013, JS Hur, XY Wang and D Liu, CH130421 (KoLRI 020827).

ACKNOWLEDGEMENTS

The work was supported by the National Research Foundation of Korea (NRF-2011-0031494) and the Korea National Research Resource Center Program (NRF-2014M3A9B8002115). DKU and SJ are indebted to the Director, CSIR-NBRI, Lucknow, India, for providing laboratory space to facilitate the identification of Chinese lichen material. SJ thanks Dr Robert Lücking for helpful suggestions.

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